Sacace BIOTECHNOLOGIES

Magno-Virus

USER MANUAL

VIRAL RNA/DNA ISOLATION KIT

REF K-2-16/1000/V

100

NAME

Magno-Virus

INTENDED USE

The **Magno-Virus** kit is designed for the rapid, efficient magnetic preparation of highly pure viral nucleic acids (e.g. HCV, HIV, HBV, HAV, HDV, Enteroviruses, CMV) from cell free body fluids such as plasma or serum.

PRINCIPLE OF ASSAY

Purification begins with the addition of Lysis reagents to the tube with the clinical sample. DNA/RNA are immobilized on magnetic particles surface and contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps using Washing Solutions. The nucleic acids can be eluted in the Specimen Diluent and are ready-for use in subsequent reactions. The prepared nucleic acids are suitable for applications like automated fluorescent RT-PCR, DNA sequencing, or any kind of enzymatic manipulation. We highly recommend the use of controls provided with the PCR amplification kit such as internal control, positive and negative controls in order to monitor the purification, amplification and detection processes.

MATERIALS PROVIDED

- **Concentrating solution** - 8 vials (14 ml each);
- **Lysis Reagent No.1**- 8 vials (4 ml each);
- **Lysis Reagent No.2**- 8 vials (7 ml each);
- **Sorbent (suspension of magnetic particles)** - 2 vial, 1 ml;
- **Solution for DNA/RNA Precipitation** – 8 vials (12 ml each);
- **Wash Solution No.1** - 8 vials (8 ml each);
- **Wash Solution No.2** - 8 vials (5 ml each);
- **Specimen Diluent** - 8 vials (3 ml each);

Contains reagents for 100 extractions from 500 µl or 1000 µl of sample.

MATERIALS REQUIRED BUT NOT PROVIDED

- Biological cabinet
- Vortex
- Tube racks
- Microcentrifuge tubes, 2,0 ml
- Magnetic separator for 2,0 ml tubes
- Dry thermal block for 2,0 ml tubes or thermo shaker
- Magnetic separator for 5,0 ml tubes (for RNA/DNA isolation from 1000 µl of plasma)
- Pipettes
- Sterile, RNase-free pipette tips with filters
- Biohazard waste container
- Disposable gloves, powderless

WARNINGS AND PRECAUTIONS

- ⚠ Component Lysis Reagents contain guanidine thiocyanate. Guanidine thiocyanate is harmful if inhaled or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/38; S: 36/37/39).

  Risk Phrases

  R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed

  R 22 Harmful if swallowed

  R 36/38 Irritating to eyes and skin

  Safety Phrases

  S 13 Keep away from food, drink and animal feedstuffs
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with Biosafety Level 2 or other appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

SPECIMEN COLLECTION AND CONSERVATION

All kind of biological fluids or semi-fluid samples can be processed e.g. serum, urine or plasma. For successful nucleic acid purification, it is important to obtain a homogeneous, clear and non-viscous sample before loading into the corresponding isolation tube. Therefore, check all samples (especially old or frozen ones) for the presence of precipitates.

Note: Handle all specimens as if they are potentially infectious agents.

RNA/DNA isolation from plasma samples:

1. EDTA tubes may be used. Follow sample tube manufacturer's instructions.
2. Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
3. Do not freeze whole blood.
4. Specimens anti-coagulated with heparin are unsuitable for this test.
5. Thaw frozen specimens at room temperature before using. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced viral titers and subsequently reduced yields of the isolated viral RNA.
6. Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.
7. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Samples containing cells, such as cerebrospinal fluid, bone marrow, urine, should first be filtered, or centrifuged for 10 minutes at 1500 x *g* and the supernatant used.

STORAGE CONDITIONS AND

- **Magno-Virus** kit should be stored dry at **+2-8°C**. The kit can be shipped at **25°C** for up to 10 days but should be stored at **+2-8°C** immediately on receipt. **Magno-Virus** reagents can be stored for up to 1 year under the above conditions without showing any reduction in performance.

PREPARATION OF WORKING SOLUTIONS

- Before use **Lysis Reagents** must be prewarmed at **60°C** for a maximum of **5 min** in order to dissolve salts.
- Vortex the vial with **Sorbent** until obtaining a homogeneous suspension. Add **140 µl** of **Sorbent** suspension into a vial with **Lysis Reagent No.2**. Mix carefully. This mix is enough for processing up to 12 samples.

  Before starting the viral DNA/RNA isolation, set the incubation block or thermo shaker at **56°C** and preheat.

PROTOCOL

1. Prepare required quantity of 2,0 ml polypropylene tubes including tubes for **Clinical Samples** and **Negative Control of Extraction**, **Positive Control of Extraction** (if provided with the amplification kit).
2. Prepare the appropriate number of vials of **Lysis Reagent No.2** with addition of **Sorbent** as previously indicated (PREPARATION OF WORKING SOLUTIONS).
3. Add **1 ml** or **0.5 ml** of each clinical sample / control to the appropriate labelled tube. Add **Internal Control** (if provided with the amplification kit) according to the manufacturer's instruction.
4. For 1 ml of clinical sample, add **1 ml** of **Concentrating solution** to each tube. In case the volume of the clinical sample is 0.5 ml, add **0.5 ml** of **Concentrating solution**.
5. Close the tubes and mix thoroughly by turning upside down 5 times and let stand for 10-15 min at room temperature. Centrifuge at 3000 rpm for 5 minutes at 18-25°C.
6. Using a new tip for each sample, carefully remove the supernatant without disturbing the sediment.
7. Add **200 µl** of **Lysis reagent no. 1** to each tube with sediment. Vortex for 10-15 seconds to resuspend the sediment. Place the tubes into a thermo shaker and incubate for 5 minutes at 56°c and 1300 rpm (alternatively place the tubes into a dry thermal block and incubate for 5 minutes at 56°C vortexing each minute). Spin shortly to collect the drops.
8. Add **500 µl** of previously prepared **Lysis reagent no. 2 with Sorbent** to each tube (already containing Lysis reagent no. 1). Vortex for 10-15 seconds. Place the tubes into thermo shaker and incubate for 10 minutes at 56°c and 1300 rpm (alternatively place the tubes into a dry thermal block and incubate for 5 minutes at 56°C vortexing each minute). Spin shortly to collect the drops.
9. Add **750 µl** of **Solution for DNA/RNA Precipitation** into each tube.
10. Vortex for 10-15 seconds. Let stand for 3-5 min at room temperature. Centrifuge at 13000 rpm for 5 minutes at room temperature.
11. Trying not to shake up the pellet, place the tubes into magnetic stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
12. Add **500 µl** of **Wash solution No. 1** to each tube. Vortex vigorously for 10-15 seconds. Centrifuge at 13000 rpm for 5 minutes.
13. Trying not to shake up the pellet, place the tubes into magnetic stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
14. Add **300 µl** of **Wash solution No. 2** to each tube. Vortex vigorously for 10-15 seconds. Centrifuge at 13000 rpm for 5 minutes.
15. Trying not to shake up the pellet, place the tubes to magnetic stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
16. Dry the pellet with open caps for 2-3 minutes at room temperature (18-25°C).
17. Add **200 µl** of **Specimen Diluent** to each tube. Vortex vigorously for 10-15 seconds. Place the tubes into thermo shaker and incubate for 10 minutes at 56°C and 1300 rpm (alternatively place the tubes into a dry thermal block and incubate for 5 minutes at 56°C vortexing each minute). Then centrifuge for 1 minute at 13000 rpm. Samples are ready for PCR or reverse transcription and PCR.

Viral RNA/DNA is stable for up to one year when stored at –20°C or –70°C.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
	Expiration Date	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**IC**	Internal Control

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